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The origin of pandemic influenza

R. G. WEBSTER [1] & W. G. LAVER [2]

Serological tests, using antisera specific to the surface subunits of the viruses (e.g., antisera to the haemagglutinin subunits devoid of any antineuraminidase or anti-host antigen activity) showed that the haemagglutinin subunits of the Hong Kong virus were, immunologically, distinct from the subunits of the preceding A/Asian strains. On the other hand, the neuraminidase subunits of the Hong Kong virus are related to those of the A/Asian strains. The haemagglutinin subunits of influenzaviruses are composed of two pairs of light and heavy polypeptide chains and it was found that the amino acid sequence of the light and heavy chains from the haemagglutinin subunits of the Hong Kong virus differed remarkably from those of the preceding A/Asian strains. These findings suggest that Hong Kong virus did not arise by mutation from a pre-existing human strain and that it probably arose by the selection of a genetic recombinant in a partially immune population. It is postulated that the recombinant was formed as the result of the mixed infection of an animal or bird with an animal or avian influenzavirus and a human A/Asian strain. The animal (or avian) virus could have donated the haemagglutinin subunits of A/Hong Kong/1/68 virus and the neuraminidase subunits could have come from the human A/Asian strain.

Particles of influenzavirus contain two different virus-coded surface antigens, the haemagglutinin and the enzyme neuraminidase. These antigens reside in different protein subunits and antigenic variation (antigenic drift and major antigenic shifts) occurs in both subunits.

Antigenic drift, which involves gradual changes in the surface antigens of influenzavirus, is thought to result from the selection, in an immune " host " population, of mutant virus particles that have altered antigenic determinants and that therefore possess a growth advantage in the presence of antibody. It has been shown that antigenic mutants isolated *in vitro* by selection with antibody have changes in the amino acid sequence in the polypeptides of the haemagglutinin subunits and it is likely that antigenic drift in the neuraminidase occurs by the same mechanisms.

The other kind of antigenic variation (major antigenic shifts) involves sudden and complete changes in one or both of the surface antigens so that " new " viruses arise to which the population has little or no immunity and it is these viruses that are the cause of the major pandemics of influenza. The origin of the " new " viruses is not known, and this communication offers speculations in the light of new immunological, biological, and chemical evidence.

In 1957 the Asian strain of influenzavirus appeared. This virus possessed haemagglutinin and neuraminidase subunits that were completely unrelated immunologically to the surface subunits of the preceding HON1 and H1N1 influenza strains. Between 1957 and 1968 antigenic drift occurred in both the haemagglutinin and neuraminidase subunits of Asian influenzavirus. Then, in 1968 a " new " virus appeared (A/Hong Kong) whose neuraminidase subunits seemed to be the same as those of the Asian strain but that had haemagglutinin subunits completely unrelated immunologically to those of the preceding Asian strains. This was shown unequivocally by using completely specific antisera to the subunits of the viruses. Thus, when antiserum made against the whole virus particle was used, cross-reactions between certain Asian strains and Hong Kong influenzavirus were obtained, suggesting that " bridging " strains between the two viruses existed. However, when specific antisera to the isolated haemagglutinin subunits of the various viruses were used (thus eliminating any contribution made by

[1] Associate Professor of Microbiology, St Jude Children's Research Hospital, Memphis, Tenn., USA.
[2] Senior Fellow, Department of Microbiology, The John Curtin School of Medical Research, Canberra, Australia.

antibody to the neuraminidase in haemagglutination-inhibition reactions) only low levels of cross-reaction could be detected between the haemagglutinins of the Asian strains and the Hong Kong virus. When the tests were carried out with viruses grown in another host (embryonated duck eggs) even these low levels of cross-reaction (probably caused by antibody to the host antigen) were eliminated and the haemagglutinin subunits of Hong Kong virus were shown to be completely different from those of the preceding Asian strains.

What was the origin of the Hong Kong influenzavirus and where did its haemagglutinin subunits come from?

There are two main theories to explain the origin of the " new " influenzaviruses. The first is that they are derived by direct mutation from existing human strains and the second is that they arise by mutation or, more likely, by genetic recombination from mammalian or avian influenzaviruses. An increasing amount of evidence is accumulating that supports the second theory. Thus, only type A influenzavirus has been found to cause human pandemics and the only strains isolated so far from mammals or birds have been of type A. Furthermore some influenzaviruses isolated from mammals and birds have been found to possess surface antigens (haemagglutinin or neuraminidase) that are identical, immunologically, to those of human type A influenzaviruses. Similarly, antibodies to surface antigens of certain human type A influenzaviruses have been found in sera from a variety of mammals and birds, both wild and domesticated. Recent studies on the chemical structure of the haemagglutinin subunits of various H2N2 influenzaviruses have suggested that the haemagglutinin subunits of Hong Kong influenzavirus were not derived by mutation from a preexisting human strain.

The haemagglutinin subunits of influenzavirus are rod-shaped structures, about 14.0 nm long and 4.0 nm wide, with a molecular weight of approximately 150 000. They are composed of two heavy polypeptide chains (M.W. about 60 000) and two light chains (M.W. about 20 000). In the intact subunits the light chain is joined to the heavy chain by –S–S– bond(s) to form a dimer of M.W. 80 000, and each haemagglutinin subunit contains two of these dimers. The two chains can be separated by SDS-polyacrylamide gel electrophoresis in the presence of 1,4-dithiothreitol or, on a preparative scale, by sedimentation through a guanidine hydrochloride and 1,4-dithiothreitol density gradient. Peptide-mapping experiments show that the two chains have quite different amino acid sequences. We have isolated the haemagglutinin subunits from a number of " old " Asian strains of influenzavirus obtained during 1957–68 and from a number of strains of " Hong Kong " influenzavirus obtained during 1968–71.

We have separated the light and heavy polypeptide chains from these subunits and each chain from each strain is being digested with trypsin and the tryptic peptides mapped. The results so far show that the polypeptide chains from the haemagglutinin subunits of the " old " Asian viruses differ greatly in amino acid sequence from the chains of the haemagglutinin subunits of the " Hong Kong " strains. Striking differences occur in both light and heavy chains.

These results are difficult to explain if the haemagglutinin subunits of the " Hong Kong " virus were derived from a preexisting human H2N2 strain. A more reasonable explanation seems to be that they came, probably by genetic recombination, from an animal or avian influenzavirus.

Genetic recombinants (antigenic hybrids) between influenzaviruses of human and mammalian (or avian) origin, as well as between human strains, can easily be produced in the laboratory by the mixed infection of cells *in vitro*. Recent experiments have also shown that antigenic hybrids can be isolated *in vivo* after the mixed infection of pigs, turkeys, and chickens with type A influenzaviruses of porcine and avian origin. Thus, chickens immunized against turkey influenzavirus haemagglutinin and fowl plague virus neuraminidase were exposed to the mixed yield from the lungs of a turkey infected three days previously with a mixture of fowl plague virus and turkey influenzavirus. These chickens died of an infection with a virus that possessed fowl plague haemagglutinin and turkey virus neuraminidase.

It has been found that the yield of recombinant viruses from mixed infections with influenzaviruses is very high, recombination frequencies of 5–50% being obtained. This is probably because the RNA of the virus genome exists in at least six discrete pieces and the high yield of recombinants during mixed infection both *in vitro* and *in vivo* may simply represent reassortment of the RNA pieces rather than classical genetic recombination.

All of these pieces of evidence taken together suggest that consideration be given to the hypothesis that " new " strains of human influenzavirus arise

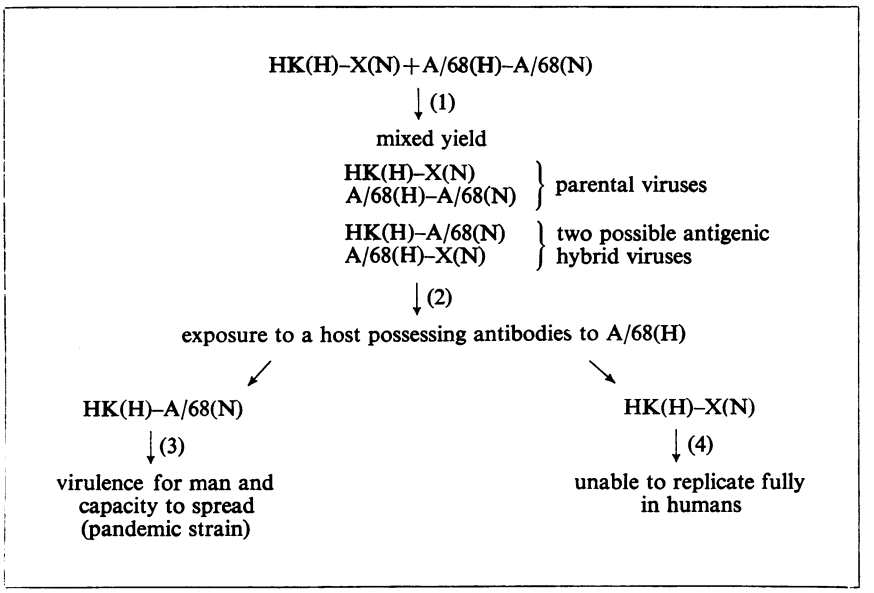

Fig. 1. Diagram illustrating how the Hong Kong strain of human influenzavirus might have arisen by genetic recombination following the mixed infection of cells with a mammalian (or avian) virus and the human A/Asian strain.

(1) Mixed infection of mammalian or avian host with viruses possessing Hong Kong (H)–X(N) (HK(H)–X(N)) and A/Asian/68 influenza (A/68(H)–A/68(N)).

(2) Transmission of mixed yield to a host animal (man) possessing antibodies to A/68(H): the parental A/68(H)–A/68(N) influenzavirus and the antigenic hybrid possessing A/68(H)–X(N) would be neutralized leaving HK(H)–A/68(N) and HK(H)–X(N).

(3) The influenzavirus possessing HK(H)–A/68(N) may be virulent for man and have the capacity to spread and become a pandemic strain.

(4) The parental HK(H)–X(N) might not be able to replicate fully in man and would be eliminated.

by genetic interaction between influenzavirus strains of human origin and mammalian (or avian) strains.

How, then, might the Hong Kong strain of human influenzavirus have arisen by genetic recombination between a human virus and a mammalian (or avian) virus? First it is necessary to postulate the existence (possibly in south-east China) of an animal or avian influenzavirus possessing the same haemagglutinin subunits as the human Hong Kong strain and neuraminidase subunits characteristic of the animal or avian strain. This virus may have been related to equine (Heq2Neq2) influenzavirus and will be designated Hong Kong(H)–X(N) where H and N are the haemagglutinin and neuraminidase subunits, respectively. Mixed infection of cells of either a human or mammalian host with this virus and the human Asian strain (designated A/68(H)–A/68(N) could have resulted in the antigenic hybrid virus, Hong Kong(H)–A/68(N) being formed by genetic recombination (Fig. 1).

It is not a requirement for such recombination that both parental viruses should be capable of complete replication in the infected cells; recombination has been found to occur even when one parent was inactivated by heat or UV irradiation before mixed " infection " of cells. Recombination has also been found to occur in animals in which only one virus replicates; thus recombinant viruses between swine influenzavirus and fowl plague virus (FPV) have been isolated from pigs—an animal in which FPV does not fully replicate. However, mixed infection of cells would need to occur and this would most probably be a very rare event. On the other hand, the emergence of " new " pandemic strains is a rare event and could be explained by this mechanism.

The mixed infection could occur in either a mammalian (or avian) or a human host having no immunity to the parental viruses. Selection of the recombinant virus having Hong Kong(H)–A/68(N) might then occur in the following way. If a host animal (possibly man) possessing antibodies to the haemagglutinin of A/68 influenzavirus were exposed to the mixed yield from an animal infected with Hong Kong(H)–X(N) and with A/68(H)–A/68(N) the Hong Kong virus (Hong Kong(H)–A/68(N) could be selected out and cause infection (Fig. 1). Thus antibodies to A/68(H) would neutralize the A/68(H)–A/68(N) parent and the antigenic hybrid possessing A/68(H)–X(N). If the parental mammalian (or avian) virus (Hong Kong(H)–X(N)) was not able to replicate in the host animal it would be eliminated. On the other hand if the recombinant virus possessing Hong Kong(H)–A/68(N) were virulent for man and had the capacity to spread, it could initiate a pandemic. Antibodies to the neuraminidase of A/68(N) would be expected to modify the severity and spread of the disease but, as was found in practice, these antibodies did not prevent the emergence of Hong Kong influenza as a pandemic strain.

ADDENDUM

Recent experiments (Laver & Webster, in press) have shown that two strains of influenzavirus isolated from horses and ducks in 1963, A/equine/Miami/1/63 (Heq2Neq2) and A/duck/Ukraine/1/63 (Hav7Neq2) possess haemagglutinin subunits that cross-reacted in haemagglutination inhibition and immunodiffusion tests with those of the Hong Kong strain of human influenzavirus A/Hong Kong/1/68 (H3N2).

Peptide maps of the heavy polypeptide chains from the haemagglutinin subunits of these three strains showed a number of differences, but maps of the light chains were almost identical, indicating that the light polypeptide chains from the haemagglutinin subunits of these animal, avian, and human viruses had practically the same amino acid sequence. These results support the suggestion that the three viruses arose, by genetic recombination, from a common ancestor.

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RÉSUMÉ

L'ORIGINE DE LA GRIPPE PANDÉMIQUE

Les auteurs examinent le problème de l'origine de la souche A/Hong Kong/1968 de la grippe humaine.

Les épreuves sérologiques pratiquées à l'aide d'antisérums dotés d'une spécificité anti-hémagglutinine exclusive (donc dépourvus de tout anticorps anti-neuraminidase) montrent que le virus Hong Kong renferme une hémagglutinine totalement distincte, immunologiquement, de l'hémagglutinine des souches asiatiques A2 précédemment identifiées. En revanche, sa neuraminidase est apparentée à celle de ces souches.

L'hémagglutinine des virus grippaux est formée de deux chaînes polypeptidiques lourdes et de deux chaînes légères. On a pu démontrer que la séquence des aminoacides dans les chaînes lourdes et légères de l'hémagglutinine du virus Hong Kong est très différente de celle qui caractérise les souches asiatiques « anciennes ».

Ces observations suggèrent que le virus Hong Kong n'est pas issu par mutation d'une souche humaine préexistante mais que, plus vraisemblablement, il résulte de la sélection, dans une population partiellement immune, d'un recombinant génétique. On peut envisager que ce recombinant a été constitué à la suite de l'infection mixte d'un mammifère ou d'un oiseau par un virus grippal de mammifère ou d'oiseau et par un virus grippal humain asiatique A2, le premier de ces virus apportant l'hémagglutinine Hong Kong et le second fournissant la neuraminidase A/68.